THE MODIFICATION OF REPRODUCTION IN INSECTS TREATED WITH ALKYLATING AGENTS. I. INHIBITION OF OVARIAN GROWTH AND EGG PRODUCTION AND HATCHABILITY

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Chemical compounds that can induce sexual sterility in the screw-worm fly (*Cochliomyia hominivorax* (Coquerel); Diptera, Calliphoridae) have received intensive study during the past two years. Such compounds have been termed "chemosterilants," regardless of their mode of inducing sterility. Chemical sterilization of screw-worm flies is of interest in a program of control or eradication of this species by the use of sexually sterilized males. Screw-worm flies have been successfully eradicated from Curaçao and the southeastern United States by the release of males rendered sexually sterile by a physical agent, ionizing radiation (Baumhover *et al.*, 1955; Knipling, 1960). The development of an effective chemical substitute for radiation might offer the additional advantages of overcoming the adverse biological effects of radiation sterilization and/or reducing the considerable mechanical handling of the flies.

Among chemical agents, biological alkylating agents appear to offer the greatest promise of success as chemosterilants against screw-worn flies (Crystal, 1963). A biological alkylating agent is a compound that can effect the addition of an alkyl group or a compound radical, with or without the replacement of a hydrogen atom, in biologically significant functional groups under physiological conditions. Various aliphatic, aromatic, and heterocyclic analogues of ethylenimine, all aziridinyl compounds, are biological alkylating agents that possess outstanding chemosterilant activity. In an earlier report (LaChance and Bruns, 1963), data were presented on the measurements of ovaries from females subjected to gamma radiation. It was found that the growth of ovaries of newly emerged females subjected to gamma radiation was much more likely to be inhibited than that in 24-hour-old or older females similarly treated. This pattern of radiation-induced sterility in female screw-worm flies forms the basis for the present comparisons of the effects of chemical sterilization. A report of the effects of selected chemosterilants on the female screw-worm fly with respect to ovarian growth and egg production and hatchability follows.

Materials and Methods

Laboratory-reared screw-worm flies are ready to mate when they are two days old, and three or four days after mating each female lays about 200–250 eggs in a shingle-like mass. The newly emerged female fly possesses a pair of immature ovaries, each consisting of 100–150 ovarioles. Maturation in all ovarioles occurs synchronously, and each ovariole produces a mature egg in the first egg mass.

In these tests each series of determinations was conducted with flies from a single rearing that emerged from 8 AM to 12 noon on the first morning of the test.

The flies were sexed and the males set aside for later use. Females less than four hours old were treated with chemosterilants the morning that they emerged; females 24 ± 2 hours old were treated the next morning. A group of females was left untreated and used as controls. The treated and control flies were caged in a colony room maintained at 80° F. and fed water and undiluted honey.

Five aziridinyl chemosterilants were used in the treatments of flies. These were: One bifunctional compound, 2,5-bis(1-aziridinyl)-3,6-bis(2-methoxyethoxy)-*p*-benzoquinone; three trifunctional compounds, thiotepa (tris(1-aziridinyl)phosphine sulfide), tretamine (2,4,6-tris(1-aziridinyl)-*s*-triazine), and methyl tretamine (2,4,6-tris(2-methyl-1-aziridinyl)-*s*-triazine); and one hexafunctional compound,

TABLE I

Growth of ovaries in 6-day-old screw-worm flies treated with chemosterilants at 0–4 or 24 hours after emergence

Chemosterilant*	Series	Mean volume (mm.³) of right ovaries with standard error from**		
		Females treated at age		Untreated
		0–4 hours	24 ± 2 hours	
Benzoquinone derivative	1	0.735±0.090 a***	4.15±0.500 b	9.39±0.435 c
	2	1.37 ±0.192 a	4.58±0.466 b	8.18±0.115 c
Thiotepa	1	4.72 ±0.640 a	6.63±0.517 b	8.51±0.374 c
	2	—	6.39±0.386 a	7.31±0.344 a
	3	6.97 ±0.482 a	8.17±0.372 a	6.29±0.429 b
Tretamine	1	2.41 ±0.288 a	7.80±0.361 b	9.12±0.330 c
	2	1.56 ±0.296 a	7.01±0.492 b	8.03±0.256 b
Methyl tretamine	1	—	5.26±0.564 a	8.28±0.397 b
	2	0.91 ±0.039 a	3.44±0.514 b	6.29±0.429 c

* Each fly treated topically on the dorsal thorax with 2 μl. of 0.5% for the first three compounds and 2% for methyl tretamine; tretamine in methanol, others in acetone.

** Twenty to thirty pairs of ovaries measured per treatment except 12 pairs in thiotepa series 1, 0–4 hours old.

*** Means within a row followed by the same letter are not significantly different from each other at the 5% level.

apholate (2,2,4,4,6,6-hexahydro-2,2,4,4,6,6-hexakis(1-aziridinyl)-1,3,5,2,4,6-triazatriphosphorine). The dosage of each compound, shown in Tables I and II, was that which had previously elicited complete or nearly complete sterility in females treated when 0–24 hours old and mated with untreated males (Chamberlain, 1962; Crystal, 1963).

Female flies were treated by the topical application of solutions of chemosterilants to the dorsal thorax with a micrometer-controlled calibrated syringe. Two microliters were applied to each fly, anesthetized by chilling, except in the first tretamine-treated group to which 2.2 μl. were delivered. In the earlier experiments, the mechanical manipulation and anesthesia of the flies, especially of the younger

TABLE II

Female fertility, fecundity, and egg hatchability data from tests with screw-worm flies treated with chemosterilants at 0–4 or 24 hours after emergence

Chemosterilant*	Series	Fertility** ($\frac{\text{♀♀ ovipositing}}{\text{♀♀ egged}} \times 100$)	Fecundity*** ($\frac{\text{Eggs/treated ♀}}{\text{Eggs/control ♀}} \times 100$)	Egg hatchability*** (%)
Treatment at 0–4 hours of age				
Benzoquinone derivative	2	0	—	—
Thiotepa	2	5	96	43
	3	53	72	40
Tretamine	2	2	73	100
Methyl tretamine	2	0	—	—
Apholate	1	70	89	85
	2	65	100	49
Treatment at 24 ± 2 hours of age				
Benzoquinone derivative	1	20	82	31
	2	35	89	3
Thiotepa	2	62	100	82
	3	71	100	79
Tretamine	2	60	100	0
Methyl tretamine	1	22	89	5
	2	21	68	0
Apholate	1	88	86	100
	2	92	100	90

* Each fly treated topically on the dorsal thorax with 2 μl. of 0.5% for the first three compounds and 2% for methyl tretamine in acetone except tretamine in methanol. Apholate: series 1, 10% in 1% aqueous Tween 20; series 2, 5% in methanol.

** Twenty-two to sixty-five females per treatment given the opportunity to oviposit.

*** Mated with untreated males at 5 days of age. Corrected for untreated controls taken as 100%; 1,500–3,000 eggs from fertilized females scored per series, except in tretamine treatment of 0- to 4-hour-old flies in which one female laid one mass of 152 eggs.

groups, resulted in severe adverse effects which were reflected in extremely poor survival. In some instances, there were enough survivors only for ovarian measurements or egg-production determinations, but not both. Therefore, in most of the later tests, great efforts were made to handle the flies very gently and to submit them to the minimum time of exposure to cold necessary for sexing treatment.

When the flies were 5 days old, males were added to the females' cages at 4 PM. The next morning, 17 hours later, the females were given the opportunity to lay eggs and the rate of oviposition and percentage of hatch were determined as previously described (LaChance and Leverich, 1962). At 6 days of age, some females from each treated and control group were killed and preserved in 70% ethyl alcohol for measurement of the ovaries. As described elsewhere (LaChance and Bruns, 1963), the product of the maximum length, width, and depth of each ovary gave an approximate volume which was sufficiently accurate for comparisons between groups.

Results

Ovarian growth

The data on ovarian measurements are given in Table I; each row represents a series of measurements of ovaries from 6-day-old females treated at the two different ages. Only determinations of right ovaries are included, since data for left ovaries were essentially identical. Differences between the mean ovarian volume of 0- to 4-hour-old flies and 24-hour-old flies, and of 24-hour-old flies and control flies, were tested for significance by the Student's *"t"* test. Those having a *P*-value of 0.05 or less have been so labeled in the table.

Reference to Table II reveals that all compounds but apholate were highly effective in their ability to inhibit egg production in very young flies. Because apholate was largely without effect as an antifertility agent in young female screw-worm flies, it was not expected that ovarian growth would be greatly inhibited. Measurements of the ovaries of females treated with this compound were, therefore, not made.

In both series of determinations with 2,5-bis(1-aziridinyl)-3,6-bis(2-methoxyethoxy)-*p*-benzoquinone, the responses of screw-worm fly ovaries were very similar (Table I). Little ovarian growth was evident when newly emerged flies were treated. Significant retardation—about 50%—of the growth of ovaries was effected by the compound when flies were treated when 24 hours old. The ovaries of flies treated at 0–4 hours after emergence were all small—50–75% of them were less than 1 $mm.^3$. Those of flies treated at 24 hours of age were distributed over a wide range of sizes; some were small, some intermediate, and some large. The measurements of ovaries of untreated flies were generally large—95% were greater than 7 $mm.^3$.

Thiotepa, tretamine, and methyl tretamine were not as uniformly effective as the benzoquinone compound. Of three replicates with thiotepa (Table I), only the first series showed a trend similar to that produced by the benzoquinone derivative. In series 1, the ovaries of newly emerged flies treated with thiotepa were about equal in size to those of 24-hour-old flies treated with the benzoquinone chemical and about half the size of those of untreated flies. The measurements of these ovaries varied widely, somewhat like those of 24-hour-old flies treated with the benzoquinone compound. Ovaries of 24-hour-old flies receiving thiotepa were intermediate in size between those of the youngest treated group and the untreated group. Measurements of ovaries of flies treated at 24 hours of age were also distributed over a wide range, but with a greater incidence of larger measurements. Nevertheless, the mean ovarian volumes of the three groups were significantly

different from each other. In series 2, survival of all flies, including males set aside for later mating, was very poor. In part, this poor survival was due to overchilling and excessive handling. It is also possible that the flies of this rearing were of low vigor, as occurs from time to time in the laboratory rearing of screw-worm flies. No young flies survived for ovarian measurements, and flies treated at 24 hours of age and control flies had ovaries very nearly alike in size. Similarly, the third series of treatments produced effects not greatly different from each other. It is thought that the small mean volume of control ovaries in this series may have been due to overcrowding in the emergence cages. Flies for treatments were removed from emergence cages as needed. The number of pupae placed in such cages varied from test to test, and the number of flies emerging may have also varied. It is suggested that the number of flies present in the emergence cage and from which the control flies were obtained was too great for the volume of the cage. Competitive stresses, greater than in noncrowded conditions, were set up which retarded the development of the control flies.

Tretamine exerted inhibiting effects on ovarian growth that were different from those produced by the first two compounds (Table I). There was a greater range of ovarian measurements with this compound than with the benzoquinone compound, but not as large as with thiotepa. At the time of the first series of tretamine tests, which was the first series chronologically, flies 48 hours old were also included. However, the mean volume of right ovaries was 8.02 ± 0.327 mm.^{3}, not significantly different from those of flies treated at 24 hours of age or of untreated flies. It was concluded that ovarian response of 24- and 48-hour-old flies in series 1 was similar, and further tests with this age group were omitted. The second series of tretamine treatments appeared to produce effects very similar to the first with respect to newly emerged and 24-hour-old flies. However, the control ovaries did not develop to the extent of those in the first replicate, perhaps due to a reduction in fly vigor or to fluctuation in environmental temperature or to overcrowding in the cages. Consequently, the means of ovarian volumes of flies treated at 24 hours and of untreated flies were not significantly different from each other.

The data of the first treatment series with methyl tretamine (2%) resemble those with the first thiotepa treatment series (0.5%) (Table I). The data of the second treatment series with methyl tretamine more closely resemble those produced by the benzoquinone derivative. Only two flies treated at 0–4 hours of age survived the first treatment; the mean ovarian volume was 4 mm.^{3}. The same general distribution of ovarian measurements was present as with the benzoquinone compound.

Egg production and hatchability

Five alkylating agents were tested for their ability to inhibit egg production in screw-worm flies treated when 0–4 hours old or 24 hours old. In some treatments, high rates of mortality, perhaps due to cold sensitivity during anesthesia, especially among newly emerged flies, resulted in reduced numbers of survivors. It is seen in Table II that the first series of treatments with the benzoquinone compound and with methyl tretamine of 0- to 4-hour-old flies are lacking. The series numbers in this table correspond to those of Table I; series numbered alike in the

two tables were treated at the same time. All of the surviving flies were used for the determinations of ovarian growth and no flies were egged in the benzoquinone and methyl tretamine series absent in Table II. The authors believe that the survivors recovered adequately from the effects of anesthesia and that the measurements of the ovaries of these flies are valid. Thiotepa-treated flies in series 1 (Table I) were subjected to stress as a result of the failure of relative humidity control, and egg production determinations of tretamine-treated flies in series 1 (Table I) were not planned. Therefore, among data for flies 0–4 hours old in Table II, there is no series 1 to relate to the correspondingly numbered series in Table I.

Females treated within four hours following emergence with the benzoquinone compound and with methyl tretamine failed to lay any eggs (Table II). Only 2% (one of 50 flies) treated with tretamine produced eggs. In one trial with thiotepa (series 2), almost all females within this age group failed to lay eggs whereas about half of those in series 3 did so. A 10% aqueous solution of apholate, which wetted the flies poorly, had the same effect as a 5% solution in methanol. Even though wetting was greatly improved with methanol, two-thirds of the flies treated at 0–4 hours of age laid eggs after treatment with either solution. Although very few flies laid eggs after some treatments with chemosterilants, those that did produced about 70% or more of normal numbers.

When screw-worm flies were treated at 24 hours of age, eggs were laid by at least 20% of the flies regardless of the chemical used. The benzoquinone compound and methyl tretamine exerted the greatest inhibitory effects on oviposition. With regard to oviposition both thiotepa and tretamine were two to three times less effective than the benzoquinone compound or methyl tretamine, and apholate was essentially without effect. In most instances, the egg production of flies was substantially normal.

Another area of influence of alkylating agents, their ability to induce dominant lethal mutations, was reflected in the hatchability of the eggs laid, as shown in the last column of Table II. When newly emerged flies were treated with the benzoquinone compound (0.5%) and methyl tretamine (2%), oviposition was completely inhibited. However, eggs laid by flies treated with these compounds at one day of age were largely nonviable, which indicated a high degree of efficiency of these compounds in inducing dominant lethal mutations in 24-hour-old flies. Thiotepa and apholate were moderately effective with newly emerged flies but largely ineffective with the older group. Tretamine was ineffective with young flies but completely effective with the older flies. Thus, dominant lethal mutations among 24-hour-old flies were extensive following treatment with the benzoquinone compound, tretamine, or methyl tretamine. The activity of biological alkylating agents as mutagenic agents in older flies is reported and discussed in greater detail in a companion report (LaChance and Crystal, 1963).

Discussion and Conclusions

The data presented above show that the effects of aziridinyl compounds on the reproductive potential of female screw-worm flies were not unlike those of gamma radiation. LaChance and Bruns (1963) demonstrated that the stage of develop-

ment of the ovarioles at the time of irradiation determined the extent to which ovarian growth was affected. The most radiosensitive stage occurred when the egg chambers contained nurse cells undergoing endomitotic replications of chromosomal material. In the female this stage corresponded to the period of adult life within four hours of emergence. When delivered during this interval of development, the same dose of radiation was much more likely to cause infecundity than when delivered to 24-hour-old adults after endomitosis was completed. With the four chemosterilants tested, the greatest inhibition of ovarian growth also occurred during the endomitotic phase of the nurse cells (0–4 hours) and resulted in complete or nearly complete infecundity. The growth of ovaries of 24-hour-old females was but slightly or moderately affected, and the fecundity of such females was correspondingly greater than that of newly emerged females. Thus, at least with respect to the effects of these compounds on ovarian growth and fecundity of female screw-worm flies, the term "radiomimetic" is quite appropriately applied.

In no instance did treatment with a chemosterilant effect a complete cessation of ovarian growth. The ovaries of normal 24-hour-old females were previously found to attain a mean volume of about 0.2 mm.^{3} (LaChance and Bruns, 1963). The smallest ovaries found among chemically treated flies were more than four times this size by the time the flies were 6 days of age. It is tempting to seek to establish a relationship between the effects of radiation (LaChance and Bruns, 1963) and those of radiomimetic chemicals. The benzoquinone compound, at a dose of 10 micrograms, produced the greatest inhibition of ovarian growth at either age of treatment. The mean volumes of ovaries from flies treated at 0–4 hours and at 24 hours after emergence were about 1 and 4 mm.^{3}, respectively (Table I). The mean volume of ovaries from 0- to 4-hour-old flies exposed to gamma radiation approached 1 mm.^{3} as the radiation dose was increased beyond 4,000 r (LaChance and Bruns, 1963). LaChance and Bruns (1963) did not report that 24-hour-old flies were irradiated, but they did expose 48-hour-old flies to various doses. It was mentioned earlier in the present report that the inhibition of ovarian growth by tretamine treatment of 24-hour-old and 48-hour-old flies was very similar. The authors believe that the response of 24-hour-old and 48-hour-old flies to gamma radiation would also be similar to each other. The mean volume of ovaries of 48-hour-old flies was not reduced below 6 mm.^{3} when flies of this age were exposed to gamma radiation between 2,000 and 8,000 r. Therefore, the treatment of 0- to 4-hour-old flies with 10 micrograms of the benzoquinone derivative or with at least 4,000 r gave about the same degree of ovarian inhibition. However, the treatment of 24-hour-old flies with 10 micrograms of the benzoquinone compound resulted in ovaries about 4 mm.^{3} in volume whereas the treatment of 48-hour-old flies with 8,000 r resulted in a mean ovarian volume of about 7 mm.^{3}. It is suggested from these results that, at these dosage levels, 24- to 48-hour-old flies were almost twice as resistant to radiation inhibition of ovarian growth as they were to chemical inhibition. Although this is a speculative conclusion based on relatively few data, it would be of interest to examine the relationship further to determine whether it is a real one. Among newly emerged flies, thiotepa was the least active chemosterilant—the mean ovarian volume in series 1 and 3 was approximately 5–7 mm.^{3} (Table I). Tretamine was the least active chemosterilant when 24-hour-old flies were treated and the mean ovarian volume of these flies

was about 7 mm.^{3}. The exposure of flies 0–4 hours and 48 hours hours of age to 2,000 r resulted in ovaries of about 4 and 7 mm.^{3}, respectively (LaChance and Bruns, 1963). Therefore, the inhibition of ovarian growth induced by thiotepa in 0- to 4-hour-old flies and by tretamine in 24-hour-old flies produced effects corresponding to those elicited by 2,000 r or less.

The following tabulation shows the relative antifertility effects previously reported (Crystal, 1963; Chamberlain, 1962) for the five aziridinyl compounds utilized in the present study. Female screw-worm flies, less than 24 hours old, were treated topically with the indicated doses of chemosterilant and mated with untreated males. The percentages of fecundity and egg hatchability have been corrected for the control percentages, taken as 100%.

Chemosterilant	Dose (μg.)	Fecundity $\left(\frac{\text{Eggs/treated } ♀}{\text{Eggs/control } ♀} \times 100\right)$	Egg hatchability (%)
Benzoquinone derivative	10	41	6
Thiotepa	12	0	—
Tretamine	11	9	0
Methyl tretamine	46	0	—
Apholate	300	75	1

At equivalent doses, the bifunctional benzoquinone derivative was incompletely effective and the trifunctional thiotepa and tretamine were equally effective as chemosterilants. Four times as much methyl tretamine as any one of these three compounds was required to produce complete sterility, whereas 30 times as much apholate was not quite completely effective. The relative inefficiency in these tests of the benzoquinone compound in preventing egg production or hatch stands in contrast to its effectiveness as an inhibitor of ovarian growth. The data reveal that the nature of the response to treatment with this chemical was dependent on the age of the fly. Only flies treated at 0–4 hours failed to lay any eggs (Table II). When older flies were treated, they laid 41% as many eggs as control flies (Crystal, 1963). Treatment at 24 hours of age resulted in 82% as many eggs being laid by treated flies as by control flies, and the ovaries of the treated flies were half as large as those of controls (Tables I and II). Tretamine is another example of a compound that was an effective inhibitor of ovarian growth in young females (Table I) but had little effect on older females, as seen in the high egg production of treated 24-hour-old females (Table II). This compound, in contrast to the benzoquinone compound, induced 100% dominant lethal mutations and was, therefore, an effective chemosterilant.

It is suggested from the data of these and other antifertility tests (Crystal, 1963) and the data of the present study that the influence of the number of reactive groups and of the nature of the molecular carrier moiety are interdependent. With respect to chemosterilization, it was observed (Crystal, 1963) that when the chemical was applied topically, compounds bearing three functional reactive groups resulted in more reliable induction of sterility than bifunctional compounds. These earlier studies also revealed that compounds with an equal number of functional groups but with different carrier moieties exerted variable antifertility effects ranging from complete sterility to fertility nearly 75% of normal.

The greatest inhibition of ovarian growth in the present study resulted from treatment with the benzoquinone derivative, a bifunctional compound. This result suggests that the quinone-ring system may have protein-associating properties if this compound is applied when nurse cells are undergoing endomitosis, and that these properties may be involved in further reaction after the molecule has been anchored to the genetic material by the alkylating aziridinyl groups. Of the two other compounds tested at 0.5%, tretamine was a more effective inhibitor of ovarian growth than thiotepa in newly emerged flies, but in 24-hour-old flies effects of both compounds were similar. Both are trifunctional but differ considerably in their carrier structures. The influence of the carrier moiety may be less important than the number of reactive alkylating groups in polyfunctional compounds such as thiotepa and tretamine. Methyl tretamine was obviously the least efficient compound of the four tested in its ability to inhibit ovarian growth. Production of similar chemosterilant and ovarian inhibitory effects required four times as much methyl tretamine as any of the other compounds. It has been previously concluded (Crystal, 1963) that the sterilizing efficacy of methylaziridinyl compounds, such as methyl tretamine, is less than that of the unsubstituted parent compounds, such as tretamine, from which they are derived.

The wide range of values obtained when measuring the volume of ovaries has been mentioned earlier in this report as an indication of the variability within groups. In general, measurements of ovaries of flies treated at 24 hours of age had a wider range of values than those of flies treated at either 0 to 4 hours of age or of untreated flies. This wider range perhaps indicated differences in physiological age as distinct from chronological age. The test insects within each series were maintained under identical conditions of environment. Nevertheless, it is probable that the rate of development varied sufficiently among individuals so that some flies were physiologically less, and some more, than 24 ± 2 hours old at the time of treatment. In this situation, treatment of flies at the terminal stages of endomitosis would inhibit ovarian growth to a greater extent than treatment after endomitosis had been completed. Greater variation in physiological development in 24-hour-old flies would be expected than in those less than 4 hours old because of the greater span of time available in which gaps in physiological age could be widened.

A study of Tables I and II reveals that flies with ovaries that had not attained a mean volume of 1.5 mm.^{3} were incapable of ovipositing. It was not until the ovaries of treated flies grew to more than 6 mm.^{3} in mean volume that at least 50% of the females laid eggs. However, there were no examples of greatly reduced fecundity among females that did oviposit. Antifertility effects in ovipositing females were expressed in the reduced hatchability of eggs as a result of the induction of dominant lethal mutations in the oocytes. No particular trend in these effects could be detected from the data in Table II. Thiotepa and apholate were somewhat more effective when newly emerged flies were treated; tretamine was ineffective at this age but completely effective when 24-hour-old flies were treated. Both the benzoquinone compound and methyl tretamine were highly effective in 24-hour-old flies, and induced large numbers of dominant lethal mutations. The data appear to justify the conclusion that the primary influence of aziridinyl compounds on the ovaries of 0- to 4-hour-old flies is the inhibition of oogenesis, and on those of 24-hour-old flies, the induction of mutations.

Summary

Female screw-worm flies (*Cochliomyia hominivorax* (Coquerel)) 0–4 hours old or 24 ± 2 hours old were each treated topically by application to the dorsal thorax of 2 microliters of a solution of one of five aziridinyl chemosterilants. At 6 days of age, some mated females were given the opportunity to lay eggs and others were killed for measurements of the ovaries. The greatest inhibition of ovarian growth occurred during the endomitotic phase of the nurse cells (0–4 hours) and resulted in complete, or nearly complete, infecundity. The growth of ovaries of 24-hour-old females was but slightly or moderately affected, and the fecundity of such females was correspondingly greater than that of newly emerged females. However, the induction of many dominant lethal mutations in 24-hour-old flies greatly reduced or eliminated the fertility of such flies with resultant sexual sterility. It was concluded that the primary influence of aziridinyl compounds on the ovaries of 0- to 4-hour-old screw-worm flies is the inhibition of oogenesis and on those of 24-hour-old flies, the induction of mutations.

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